

Examples

General methods

Fermentation broth from *Propionibacterium freudenreichii* CBS 929.97 was
5 obtained as described in International Patent Application WO00/37699.

The fermentation broth was concentrated by means of ultrafiltration (on polysulfon
MW cut off 5-10 kD, Koch HFK 151 VSV) or microfiltration (on Membralox ceramic
0.1µm) up to a biomass concentration of 100-150 g/l.

After ultrafiltration or microfiltration, the propionic acid in the biomass had a
10 concentration of about 25-30 g/l. To reduce the concentration of propionic and acetic acid
in the biomass, the biomass concentrate was diafiltered with water. This diafiltration was
performed by an in-line addition of water to the concentrate at the same rate as the
permeate flow. The diafiltration was stopped at a propionic acid concentration lower than 5
g/l. At this purpose a ratio (v/v) water : concentrate of 3-4 : 1 was applied.

15 After diafiltration the concentrated biomass was pasteurised during 1 minute at a
temperature of 90-940C (either by direct steam injection or heating by a plate heat
exchanger).

The pasteurized biomass was further concentrated by a multistage (vacuum) falling
film evaporator with vapor recompression. This type of evaporator is known to those
20 skilled in the art.

The following conditions were applied.

Biomass feed rate	2000-3000 l/h (corresponding to 300 kg dry matter/h)
25 Pre-heater temperature	920C
1st stage temperature	65-700C
5th stage temperature	50-550C
Temperature of concentrate	45-500C
Biomass concentration	22-26% (1250 kg/h)

The biomass concentrate was spray-dried on a Multi Stage Dryer (NIRO AS, Denmark).

The following set up was used in all the experiments.

The vitamin B12-containing biomass was fed into the drying chamber by a nozzle
5 with a biomass feed rate of 1250 kg dry matter/h).

Nozzle pressure 190-195 bar

Air inlet temperature (co current) 200-2200C

Air outlet temperature 75-920C

Air Internal fluid bed temperature 55-600C

10 Air 1st external fluid bed temperature 30-350C

Air 2nd external fluid bed temperature 15-200C

Powder temperature < 300C

Fines were returned via a cyclone to the nozzle area.

15 **(Comparative) Example 1**

In this example vitamin B12-containing biomass was spray-dried in absence of solid carrier applying the above-mentioned spray-drying conditions.

Example 2

20 In this example 600 kg of vitamin B12-containing spray-dried biomass obtained in Example 1 was mixed in an external powder mixer (batch) with 300 kg of wheat flour and 1 kg of silica (Aerosil 200®).

Example 3

25 In this example 120 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ per kg of concentrate was added to the diafiltered biomass concentrate (120 g/l biomass concentration) before evaporation. The mixture was evaporated to a dry matter content of 32% and spray dried as described above.

Example 4

30 In this example vitamin B12-containing biomass was spray-dried in presence of wheat flour as a solid carrier applying the above-mentioned spray-drying conditions. The



INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 20897WO		FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/12701	International filing date (day/month/year) 13.11.2003	Priority date (day/month/year) 14.11.2002
International Patent Classification (IPC) or both national classification and IPC C12P19/42, A23L3/46		
Applicant DSM IP ASSETS B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
 - ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:
 - I ☒ Basis of the opinion
 - II ☐ Priority
 - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - IV ☐ Lack of unity of invention
 - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - VI ☐ Certain documents cited
 - VII ☐ Certain defects in the international application
 - VIII ☐ Certain observations on the international application

Date of submission of the demand 09.06.2004	Date of completion of this report 07.12.2004
Name and mailing address of the International preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Popa, M Telephone No. +49 89 2399-7829 

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. **PCT/EP 03/12701**

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17):*

Description, Pages

1-14, 17	as originally filed
15-16	received on 27.10.2004 with letter of 25.10.2004

Claims, Numbers

1-24	as originally filed
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2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. **PCT/EP 03/12701**

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability;
citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes: Claims	
	No: Claims	1-24
Inventive step (IS)	Yes: Claims	
	No: Claims	1-24
Industrial applicability (IA)	Yes: Claims	1-24
	No: Claims	

2. Citations and explanations

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/EP 03/12701

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The following documents (D) are referred to in this communication:

D1: GB-A-1 321 702 (RICHTER GEDEON VEGYESZETI GYAR RT.) 27 June 1973

D2: WO 98/06868 A (GIST BROCADES BV) 19 February 1998

D3: DATABASE WPI Section Ch, Week 199409 Derwent Publications Ltd., London, GB; Class B02, AN 1994-072960 XP002236803 & RU 2 001 953 C (ANTIBIOTICS ENZYMES RES TECHN INST) 30 October 1993

D4: FR-A-2 802 212 (AGRONOMIQUE INST NAT RECH) 15 June 2001

2. Words like *preferably* are considered to have a non-limiting effect upon the scope of the affected claim, namely 7. Therefore, the features introduced by such words are disregarded in the analysis of submitted claims.

The amendments brought by the applicant comply with the requirements of Art. 34(2)(b) and R. 91:1(b) PCT.

3. The present application does not meet the requirements of Article 33(2) PCT, because the subject-matter of the independent claims **1, 9, 20, 22** and **23** is not novel.

3.1. Document **D1** discloses (the references in parentheses applying to this document) a dry powder made of a combination of vitamin B₁₂-containing biomass (propionibacteria and/or methanobacteria) and a carrier (proteins and/or carbohydrates) intended to be used directly as a feed supplement or, after subsequent purifications, for human therapeutical aid (page 2 lines 26-86). Among suitable carriers, starch has been explicitly mentioned (page 2 lines 46-53). In Example 2, potato starch (=potato flour) was used as carrier.

As a consequence, claims **1** and **20-24** are not allowable under Art. 33(2) PCT for lack of novelty in its subject-matter.

- 3.2.** Document **D2** also discloses a method for producing particles comprising vitamin B12-containing biomass and a solid carrier comprising the step of spray drying the biomass and the carrier (p. 6 l. 10-16; p. 7 l. 11-14; p. 8 l. 12-21).

Note In view of the substantive examiner, the granular product obtained by drying a broth containing biomass and carbohydrates and/or proteins as nutritive medium will inherently satisfy all the features of the independent claim **1** and some of its dependants (depending on the actual composition and bacteria used). This is the case of all the documents mentioned under X category in the Search Report.

- 4.** For the sake of completeness, the applicant is informed that the rest of the features found in the dependent claims involves no inventive steps, provided these features may be found novel over certain prior art, as they are obvious for the skilled person or represent routine laboratory work.